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The *TREX1* Dinosaur Bites the Brain Through the LINE

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Abstract:

In this issue, Thomas et al. (2017) define the nature of accumulated ssDNA present in the neuron and astrocyte cytoplasm of *TREX1* mutated stem cell-derived organoids. Accumulated ssDNAs are derived from LINE-1 endogenous retroelements, providing new clues as to the development of Aicardi-Goutières syndrome in the neural system.

Aicardi-Goutières syndrome (AGS) is a rare recessive neurological brain disease caused by mutations in one of several genes, among which three-prime repair exonuclease I (*TREX1*) was first identified. This finding opened a new avenue of research, as *TREX1*' 3'- 5' exonuclease activity proved important in limiting the cellular accumulation of nucleic acids, such as single stranded DNA (ssDNA), which activates innate immune responses to viruses and the production of type I interferon that may lead to the development of systemic lupus erythematosus (SLE)-like symptomatology.

Long INterspersed Element class 1 elements (LINE-1 or L1 retroelements) are very abundant in most mammalian genomes and comprise more than 20% of human and mouse genomes (Garcia-Perez et al., 2016). However, and despite their abundance, only a small fraction of these LINE-1 sequences are currently active. LINE-1s are highly active in the nervous system, and *TREX1* is one key regulatory protein known to inhibit the overactivity of LINE-1, suggesting that endogenous retroelements could be linked to the development of AGS. It has been suggested that endogenous retroelements may play a role in the development of autoimmunity and particularly in SLE (Perl et al., 2010). Stetson and colleagues (Stetson et al., 2008) showed that *TREX1* could metabolize ssDNA derived from endogenous retroelements that accumulate in *Trex1* deficient mice. They also demonstrated how *Trex1* controls the retrotransposition rate of LINE-1s. Similarly, a more recent study also demonstrated that *SAMHD1*, a gene also involved in AGS, controls the rate of human LINE-1 retrotransposition in cultured cells (Zhao et al., 2013), although the mechanism is not completely clear.

In this issue, Thomas et al. (Thomas, 2017) further clarify the connection between endogenous retroelements and AGS. They develop a human stem cell-derived cortical organoid model using pluripotent stem cells derived from patients with AGS or produced using CRISPR/Cas9 genomic editing to introduce damaging mutations in the *TREX1* gene. In addition, the organoids help the authors to closely analyse the mutations' effects in neural tissue, a target of the disease, allowing the dissection of the role of various cell types. Furthermore, a major finding of this study is the demonstration that ONLY active LINE-1 elements are indeed related to AGS pathology. Active LINE-1s are non-LTR retrotransposons that move by a copy and paste mechanism, using an intermediate RNA and Reverse Transcriptase Activity (Garcia-Perez et al., 2016), in a process known as retrotransposition. To do that, mammalian LINE-1s encode two key enzymatic activities: ENdonuclease (EN) and Reverse Transcriptase (RT) (**Figure 1A**), and both activities are required for their mobility in generating new genomic insertions distributed randomly in genomes (**Figure 1B**).

Thomas and colleagues provide compelling evidence suggesting that active LINE-1 elements generate abundant cytoplasmic LINE-1-derived single stranded DNA (ssDNA) molecules in *TREX1* mutant cells. Although this finding is supported by the use of chemical inhibitors of the LINE-1-encoded RT and genetic approaches to reduce LINE-1 activity, major mechanistic questions remain

unexplored. It is not clear whether cytoplasmic ssDNAs are indeed generated in *TREX1* mutant cells. A likely scenario is that these ssDNAs are abortive LINE-1 retrotransposition intermediates (**Figure 1B**) that might gain access to the cytoplasm; these abortive LINE-1 intermediates might be over-produced in the absence of *TREX1* activity, which normally might act to titrate the number of new LINE-1 insertions that a cell would normally accommodate. However, it is also possible that these ssDNAs are generated directly in the cytoplasm of *TREX1* mutant cells, although it is not clear how these ssDNAs might be primed. Either way, it is clear that these ssDNAs generated by active LINE-1s are directly implicated in the pathophysiology of *TREX1* mutant cells, and presumably of AGS patients. This data is remarkable, as it provides the first clear demonstration that active LINE-1s, and only active LINE-1s, might be directly related to human AGS pathology. In addition, these data might help clarify why mouse models of AGS do not recapitulate the major symptoms observed in human AGS patients; it is tempting to speculate that as mouse cells normally deal with an excess of active LINE-1s (approximately 3000 active LINE-1s/mouse cells), they have greater tolerance to cytoplasmic ssDNAs before eliciting an autoimmune reaction than human cells, which normally deal with just 80-100 active LINE-1s/human cell.

These results have additional important implications. For the first time, a tissue specific effect of *TREX1* is observed where on the one hand, neurons become importantly affected and suffer apoptosis, something that does not occur in neural precursors or in astrocytes (Thomas, 2017). However, and importantly, astrocytes produce type I IFN following the accumulation of LINE-1-derived ssDNA, which increased the toxic effects to which neurons were subjected.

Clinically, this study not only provides a plausible explanation for the microcephaly observed in AGS, but also provides hints as to the development of neuropsychiatric lupus. Patients with AGS caused by any of the genes with damaging mutations have increased activity of type I interferon in the cerebrospinal fluid, and patients rapidly develop neurological disabilities (Crow et al., 2015). Although contrary to previous belief, it has now been accepted that a larger proportion of patients with SLE show neuropsychiatric manifestations. In fact, neuroimaging evidence suggests that early cases of SLE, without neurological symptomatology, suffer from glucose hypometabolism consistent with early apoptosis or atrophy in portions of the frontal and parietal cortex, as well as inflammation (Ramage et al., 2011). It would be of interest to investigate if there is a relationship between *TREX1* heterozygous mutations and the presence of apoptosis and atrophy changes early in these patients. Furthermore, in general, neuroimaging monitoring of SLE patients with *TREX1* mutations may suggest the excessive production of type I IFN activity in the brain, and analysis of cerebrospinal fluid to show this, might be recommended in the clinic. *TREX1* may lie behind a larger proportion of neuropsychiatric SLE (Fredri et al., 2015) patients, who in addition with AGS patients, would be good candidates for very early anti-type I IFN receptor treatment currently in clinical trials that may help them avoid critical brain damage.

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Figure legend

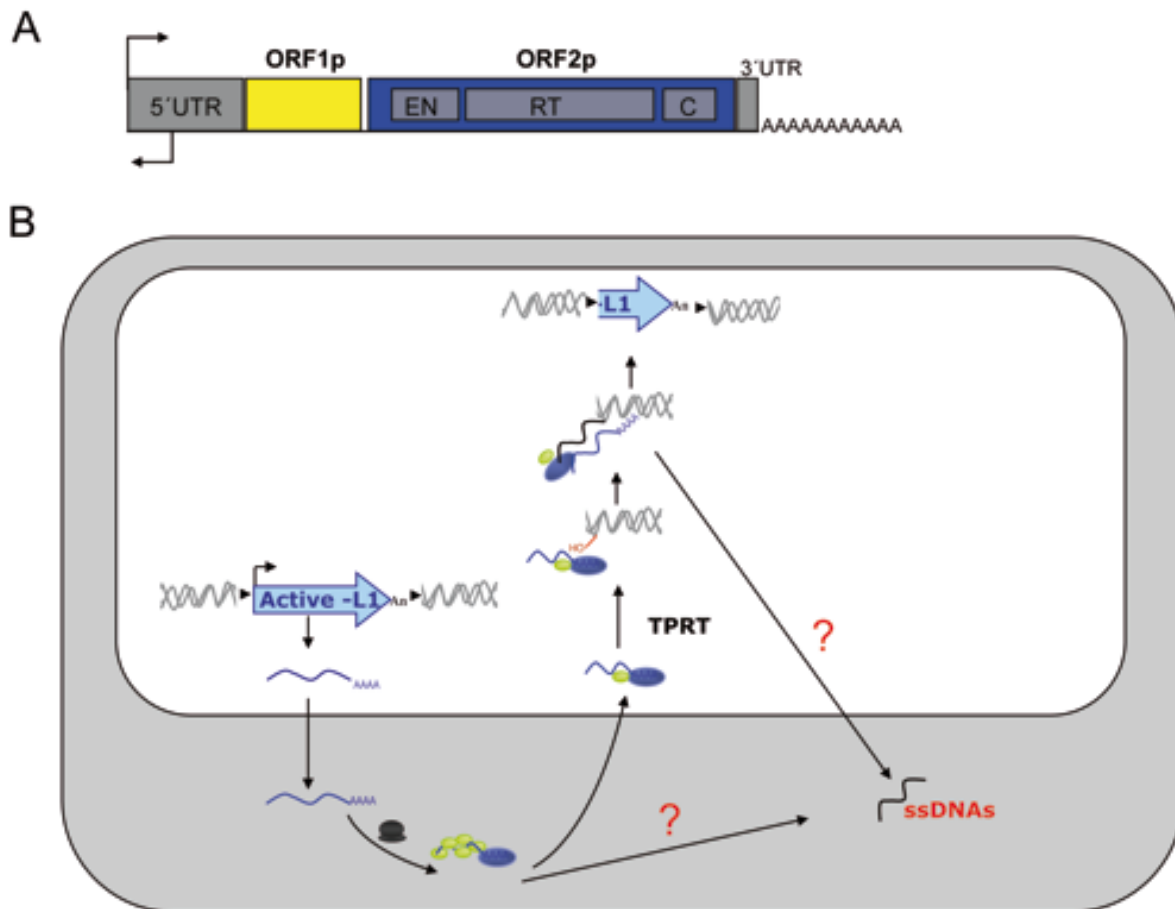


Figure 1. A. Structure of a human active LINE-1 element. The relative position of the 5'UnTranslated Region (5'UTR, grey box), ORF1p (yellow box), ORF2p (blue box) and 3'UTR is indicated. Within ORF2p, the relative position of the endonuclease (EN), reverse transcriptase (RT), and cysteine-rich (C) domain are also indicated. **B. One round of LINE-1 retrotransposition by Target Primed Reverse Transcription (TPRT).** The endonuclease (EN) and reverse transcriptase (RT) enzymatic activities of LINE-1 are encoded within Open Reading Frame 2 (ORF2) of active LINE-1 elements, and both activities are strictly required for successful LINE-1 retrotransposition. Upon transcription and translation, both LINE-1-encoded proteins (ORF1p and ORF2p) bind back to their encoding mRNA by a process termed *cis*-preference, and this complex gains access to the nucleus. Once in the nucleus, the EN activity of LINE-1 processes the bottom strand, releasing a 3'OH that is used by the RT activity to generate the first cDNA strand of the new insertion, already linked to the genome.